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# The Breeding Ecology and Natural History of Ambystomatid Salamanders in an Ephemeral Wetland in Mason County, West Virginia

S. Douglas Kaylor

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**The Breeding Ecology and Natural History of Ambystomatid  
Salamanders in an Ephemeral Wetland in Mason County,  
West Virginia**

**Thesis submitted to  
The Graduate College of  
Marshall University**

**In partial fulfillment of the  
requirements for the degree of  
Master of Science  
in Biological Sciences**

**by  
S. Douglas Kaylor**

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Dr. Charles Somerville**

**Marshall University  
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## Abstract

A forested ephemeral wetland in Mason County, WV, documented to contain 4 of 5 *Ambystoma* species found in the state, was studied to learn about population sizes, breeding cycles, and habitat use. Minnow traps were placed along three drift fences with additional traps placed throughout the study area. From February 5 to March 27, 2005, 85 captured adults were identified to species, marked by elastomer injection for mark-recapture analysis, and measured for morphometrics. Larval salamanders were identified, staged, measured, and returned. Egg clutches were mapped and counted. Mark-recapture analysis suggests the *A. texanum* population size is between 635 and 735 individuals (95% confidence). No other species of *Ambystoma* were marked though several *A. opacum* larvae were trapped, and three *A. opacum* adults were observed and measured during Fall 2005. Despite the absence of other species, this breeding habitat is crucially important to the conservation of *A. texanum* in West Virginia.

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# Table of Contents

Chapter 1: Introduction .....	1
Amphibian Decline .....	1
Importance of Amphibians and Salamanders .....	1
Description of Study Area .....	2
Family <i>Ambystomatidae</i> .....	3
Species Descriptions .....	3
Research Objectives .....	5
Chapter 2: Materials and Methods .....	6
Trapping .....	6
Morphology .....	7
Mark/Recapture .....	7
Statistics .....	8
GIS Map Generation .....	9
Chapter 3: Results .....	11
Ambystoma Found and Mark/Recapture Analysis .....	11
Migration .....	11
Morphometrics .....	11
Breeding Behavior .....	13
Clutch Size/Location .....	13
Larval Development .....	13
Mutations .....	13
Chapter 4: Discussion .....	14
Ambystoma Found .....	14
Morphometrics .....	15
Migration .....	16
Breeding Behavior .....	17
Clutch Size/Location .....	17
Mutations .....	18
Final Conclusions .....	20
Literature Cited .....	21
Appendix 1: Figures .....	25
Appendix 2: Tables .....	37
Appendix 3: Listing .....	42

# Chapter 1: Introduction

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## Amphibian Decline

Scientists first became concerned about wide-spread amphibian population declines in 1989 at the First World Congress of Herpetology. Since that time, amphibian populations worldwide have been in decline. Based on data published in 2004 by the World Conservation Union (IUCN) in the Global Amphibian Assessment, out of all currently known amphibian species (5709 species) at least 2468 amphibian species are experiencing some form of population decrease; whereas 1552 are stable and only 28 are known to be increasing. Due to a lack of data, 1661 species have an unknown trend. Figure 1 shows the percentage values for this data. In addition, IUCN lists 427 of all amphibian species (7.4%) as Critically Endangered, the highest category of threat (Stuart et al 2004).

There are six major factors for this alarming decline: overexploitation, urbanization, introduced predators, chemical pollutants, global change (including UV radiation and climate change), and disease. Of these factors, chemical pollutants, urbanization, and disease are the three most influential in the Ohio River Valley in West Virginia. Many of the original wetlands along the Ohio and Kanawha Rivers have been urbanized and valuable amphibian habitat has been lost. Redleg disease (*Aeromonas hydrophila*) has been documented among *Rana pipiens* in the Greenbottom Wildlife Management Area in West Virginia (Sutton 2004), and several mutations have been documented in frogs and salamanders from the same area and surrounding counties (Sutton 2004, Fiorentino 2002).

## Importance of Amphibians and Salamanders

The diverse ecological roles of salamanders in natural areas underscore the importance of their conservation. Permeable skin, gelatinous eggs, and gilled larvae make amphibians vulnerable to soil and water pollution. Salamanders serve as a cost-effective and readily quantifiable measure of ecosystem health and integrity.

In addition to being important indicator species, amphibian species contribute greatly to ecosystem function. Through association with underground burrow systems, they contribute to

soil dynamics. In many systems, salamanders operate as keystone predators, fulfilling much the same role as fish in other ecosystems. As mid-level vertebrate predators, they provide direct and indirect biotic control of species diversity and ecosystem processes along grazer and detritus pathways. Wyman (1998) studied the effect of salamander predation on detrital food webs, particularly focusing on invertebrates, decomposition and the carbon cycle. He found that predation by salamanders affected the composition of leaf litter and humus invertebrate communities. Salamanders also indirectly affected decomposition rate by preying on leaf litter fragmenters. He found that salamander presence reduced the rate of decomposition by between 11 and 17%, thus exhibiting top-down control on decomposition processes.

Salamanders are an important part of the energy flow between freshwater and terrestrial ecosystems. High in protein and lipids, they supply high-quality and slowly available stores of energy and nutrients for tertiary consumers. Up to 60% of the energy they consume is passed up the food chain (Burton and Likens 1975). Salamanders connect energy cycles between aquatic and terrestrial landscapes. Regester et al. (2006) studied the energy flow and energy subsidies associated with seasonal migrations and emergences of *Ambystoma opacum* (Marbled salamander), *A. maculatum* (Spotted salamander) and *A. tigrinum* (Tiger salamander) in forested ephemeral ponds in southern Illinois in 2002 and 2003. They found oviposition by female Ambystomids added up to 5.5g ash free dry mass (AFDM) m<sup>-2</sup> year<sup>-1</sup>. The entire larval assemblage produced as much as 7.9g AFDM m<sup>-2</sup> year<sup>-1</sup> (Regester et al. 2006).

## Description of Study Area

The site of the study is a forested ephemeral wetland in the flood plain of the Ohio and Kanawha Rivers. It is located on the property adjacent to Moose Lodge #731 in Point Pleasant, Mason County, West Virginia, along West Virginia Route 2 North. The wetland has several long parallel furrows (Figures 2 and 3), the reasons for which have yet to be determined. These depressions filled with water in late October 2004 and ponds dried up in early June 2005. As the summer was a dry one, the ponds did not fill up with water again until early December 2005.

Tree species found in the study area include *Quercus palustris* (Pin Oak), *Liquidambar styraciflua* (Sweet Gum), and *Acer saccharum* (Sugar Maple). These trees provide large quantities of leaf litter and deadfall, which form refugia for larval and adult salamanders as well

as the macroinvertebrates upon which they feed. *Rhus radicans* (Poison Ivy), *Rosa multiflora* (multiflora rose) and *Smilax spp.* (Green Briar) dominate the shrub layer. A large variety of fauna were encountered at the study site during the period of the study. Of particular note are *Vulpes vulpes* (Red Fox), *Chelydra serpentina serpentina* (Snapping Turtle), Great Blue Heron, *Procyon lotor* (Northern Raccoon), *Rana clamitans melanota* (Green Frog), *Thamnophis sauritis sauritis* (Eastern Ribbon Snake), *Lepomis macrochirus* (Bluegill) and *Orconectes rusticus* (Rusty Crayfish) all potential predators of salamanders.

## **Family Ambystomatidae**

As one of the six families of advanced salamander, Ambystomatids are characterized by the lack of nasolabial grooves, the possession of large functional lungs, a stout body and limbs and a thick tail. Three species of salamanders belonging to the Genus *Ambystoma* have historically been found in the study area: *A. jeffersonianum* (Jefferson's Salamander), *A. opacum* (Marbled Salamander), and *A. texanum* (Small-Mouthed Salamander). It is likely that *A. maculatum* (Spotted Salamander) occurs in the area as well, as its distribution is statewide and breeding ponds like those found in the study site are commonly utilized .

## **Species Descriptions**

*Ambystoma jeffersonianum* can vary in color from a dark brown, to brownish gray to slate gray and may have light blue speckles scattered along the sides, tail, and occasionally extending onto the back. It has relatively long, slender limbs and toes compared to other Ambystomids, and the tail is laterally compressed and is almost as long as the body (Green and Pauley 1987)(Figure 4). Average adult length ranges from 10.7 to 21 cm, with females being in the upper part of the range. Breeding males have swollen vents and appear more slender than gravid females. The tail is also longer and more laterally compressed in males. Larvae are a yellowish green color with dark blotches on the back. They possess a relatively uncolored caudal fin and display external gills upon hatching. Older larvae have a mottled greenish gray dorsum and may be marked along the sides with small yellowish spots while the ventrum is pale and generally unmarked (Petranka 1998). *Ambystoma jeffersonianum* is listed as a species of special concern class 3 in West Virginia. Green and Pauley (1987) state that its distribution is probably statewide but note that



most records are from southeastern counties or the eastern panhandle. In 2003 William Sutton found one *A. jeffersonianum* in the research site under a rotten log (W. Sutton, pers. comm.)

*Ambystoma opacum* attains an adult length of approximately 9-10.7 cm and has white or light gray crossbands across the head, back, and tail (Conant and Collins 1998). Sexually dimorphic males have silvery white crossbands, which become very white along with swollen cloacal glands, during the breeding season in early autumn. The female is larger and possesses silvery gray crossbands (Petranka 1998) (Figure 5). Unlike other species of *Ambystoma*, *A. opacum* mates in the fall usually in late September (Green and Pauley, 1987). After mating females nest usually in a reduced pond, or dried bed of a temporary pond or ditch where they lay between fifty and one hundred eggs. Once the eggs are deposited, females remain with the eggs to keep them moist until nests are flooded. As soon as the autumn rains come eggs hatch in the depression where they were originally laid. If rain does not come and temperatures do not fall too low, the eggs will over-winter and hatch the following spring (Petranka 1998). Because of fall breeding, larvae collected in the early spring are normally much larger than other *Ambystoma* larvae that share the same breeding ponds, resulting in an ecological advantage.

In comparison with other members of the genus, *A. texanum* has a relatively small head with a blunt, short snout. The head tends to appear swollen behind the eyes and the lower jaw barely protrudes past the upper jaw. Coloration of the dorsum varies from pale gray to black with an irregular pattern of light blotches on the upper surface of specimen. The light pattern becomes darker on the sides and extends to a dark belly (Conant and Collins 1998)(Figure 6). Adult length is normally between 11-17.8 cm, with 14 to 16 costal grooves. Males are smaller with longer and more compressed tails. Larvae usually have light bars or crossbands on an olive green or dark brown background. In the winter of 2004 a new population of *A. texanum* was discovered at the research site. *A. texanum* is listed as a species of special concern class 1 in West Virginia, meaning that there are five or fewer documented occurrences, or very few remaining individuals, within the state. Species that are classified in this group are extremely rare and critically imperiled or especially vulnerable to extirpation. It has a limited distribution in West Virginia and has only been found in a few sites in Wood and Mason counties.

*Ambystoma maculatum* is a rather large salamander ranging in length from 11-24 cm. Adults are black to gray with two rows of distinct, round yellow or orange spots running lengthwise down the back. The rows of spots may be either irregular or straight and run from the eyes to the tail tip. The belly and lower sides are slate gray and lighter than the dorsal ground color (Conant and Collins, 1998) (Figure 7). Eggs are laid in large masses that adhere to submerged branches; these masses contain up to 200 eggs surrounded by a conspicuously thick, firm, jelly-like matrix (Petranka 1998).

## **Research Objectives**

This study was designed to determine which *Ambystoma* species are present in the wetland and the sizes of their respective populations. Additionally the study examined details on their Ambystomatid breeding cycles and behavior. I gathered data to characterize the habitat usage by species, looking at where eggs are laid and attempting to study migration patterns. Lastly, morphometric data was collected and mutations and malformations were noted in order to collect data about the health of the salamander populations present.

## Chapter 2: Materials and Methods

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### Trapping

During the winter of 2003-2004 a few initial wire circular funnel traps were placed in the wetland as part of a yet unpublished study to find suitable habitat for, and possibly more populations of, both *Ambystoma barbourii* (Streamside salamander) and *A. texanum* (Unpublished Data, Stewart and Loughman). On the night of March 1, 2004, *A. texanum* were observed breeding. A detailed account of breeding behavior was written upon return to the lab and is included in this report.

General methodology to study use of a wetland by migratory amphibians includes completely surrounding the wetland with drift fencing and then placing trap arrays along the outside and inside perimeters of the fencing. The study animal is then trapped upon entering, transferred into the wetland by the researcher and then it is trapped again when leaving. This methodology was not attempted due to the size of the wetland. Alternatives are random searches, which require many search hours and many trained investigators, and random sample trapping. These methods were employed in the initial trapping of the wetland before the study began.

Wilson and Dorcas (2004) conducted a study comparing the effectiveness of aquatic drift fences with traditional funnel trapping as a quantitative method for sampling amphibians. They found that using silt fencing with rectangular funnel traps, as opposed to the traditional method of placing minnow traps directly in the water, greatly improved amphibian capture rates. Traps with fencing captured significantly greater numbers of larval *A. opacum* ( $p < 0.001$ ) and larval *Pseudacris triseriata*, western chorus frog, ( $p = 0.001$ ) as well as capture rates for a large variety of adult amphibians. Traps with fencing also captured significantly more life stages or more species per trap than unfenced traps ( $p < 0.001$ ). Finally cost and time invested per amphibian captured were substantially lower when traps were used in conjunction with drift fences.

Both solitary randomly-placed collapsible rectangular mesh minnow traps (model RN10; Memphis Net and Twine Co. Inc., Memphis TN) were used as well as the same model traps used in conjunction with prefabricated 50' silt fencing. On February 5, 2005 ten initial solitary traps were placed in the study area, with an additional six in an adjacent site. Three drift fences with 8

funnel traps each were placed February 12, 2005, when ice was sufficiently thin to allow placement (Figures 8, 9 and 10).

## **Morphology**

Upon capture, measurements of each salamander were performed using plastic vernier calipers accurate to 0.1 mm. Snout-vent length (SVL), tail length, and cranial width were measured for each adult salamander. Weight was measured with 30 gram Pescola scales accurate to 0.1 g. Measurements were recorded in millimeters and grams respectively, and double-checked for accuracy. Larval salamanders were identified to species with Dr. Tom Pauley from Marshall University verifying identification. Larvae were then measured, staged, and then released at the point of capture. Egg masses were identified to species, mapped and counted. Where egg masses were laid in traps, they were noted, counted and placed on substrate in the water nearby.

## **Mark/Recapture**

Adult salamanders captured were marked for mark/recapture analysis using visible implant elastomer. A two part silicone based material is mixed and then injected under translucent or transparent skin which then hardens into a pliable biocompatible solid. The tags fluoresce under blue light. This technique is useful on small specimens, has minimal impact on survival growth and behavior, and retention rates of the marks are high (Wooley 1973).

Adult salamanders captured within the main study site were marked under the dermis on the dorsal side of the left front shoulder joint (Figure 11). Adult salamanders captured from the secondary site were marked under the dermis on the dorsal side of the right front shoulder joint. After marking, adult salamanders were released at the point of capture. One individual from the supplementary site was kept in a terrarium in the lab for three months to test for mark retention. Upon completion of the three-month period the mark was still highly visible. Larval salamanders were too small to mark. Owing to the difficulty in marking small numbers of individuals, four single individuals encountered on random searches were also not marked.

## Statistics

An initial estimate of population size was generated from mark-recapture data using the refined Lincoln-Peterson method, a revised version of Lincoln-Peterson that corrects for sampling bias:

$$N = \frac{(n_1 + 1)(n_2 + 1)}{(m + 1)} - 1 \quad \text{Eq. 1.}$$

where  $N$  is the total population,  $n_1$  is the number marked on the first visit,  $n_2$  is the number captured, and  $m$  is the number of recaptures.

The standard deviation of this population estimate was also calculated:

$$StdDev(N) = \sqrt{\frac{(n_1 + 1)(n_2 + 1)(n_1 - m)(n_2 - m)}{(m + 1)^2(m + 2)}} \quad \text{Eq. 2}$$

The revised Lincoln-Peterson method gives a population estimate on a normal distribution. Mark-recapture events, however, follow a Poisson distribution with:

$$N = \Phi(\lambda) + n_1 \quad \text{Eq. 3}$$

$$\lambda = n_1 \left( \frac{n_2}{m} - 1 \right) \quad \text{Eq. 4}$$

where  $\lambda$  is the mean of occurrences (in this case total population size) and the mode of the probability distribution.

To make the Poisson distribution a highly accurate estimate of population size, a maximum population size was applied to the distribution as an informed prior. Four standard deviations from the Lincoln-Peterson estimated mean (99.99% confidence) was calculated. This maximum was expressed as an exponential distribution. The composite probability distribution function was determined using a Gibbs sampler implemented in MatLab (Appendix 3).

Morphometric data collected from individuals were plotted against each other to determine if some metrics were predictors for some other. A linear least squares fit and  $R^2$  values were calculated for each pairing using SigmaPlot 10.0.

## **GIS Map Generation**

The tabular data collected has been related to a map of the wetland generated using ArcGIS. This map helps to organize and conceptualize data that can be queried and presents data in a clearer manner than data tables and charts. The map also provides a clearer understanding of microhabitat usage by these salamanders, distribution within the wetland, and sites of egg laying.

The raster images included are hillshade relief, topography maps of the study site, and aerial photos of the site. Raster images from both the Beech Hill quadrangle and the Gallopolis quadrangle were added, because the study site is found along the edge of these two quadrangles. The hillshade relief layer (created by combining a 70% transparent elevation layer with the hillshade layer) shows the elevation and the land contours of the study site. This layer also indicates that the entire study site is located within the floodplain of the River, which reaches beyond the Point Pleasant Moose Lodge. The USGS topography maps used are in standard scale (1:24,000). The topography maps of Beech Hill and Gallopolis show municipal structures such as buildings, roads, gas wells, and powerline cuts. Lastly, DRG aerial photographs for Beech Hill (northwest quadrant) and Gallopolis (northeast quadrant) were added. These surfaces provide important information on microhabitat within the wetland including the areas that are underwater during the use of this wetland and vegetation type.

Vector images included as part of this project are the West Virginia counties shapefile and the West Virginia wetland layer. Vector data sets were also developed as part of this project. The locations of traps were recorded with a Trimble GPS unit and then the UTM coordinates were uploaded to an Access database using GeoExplorer. These points were then transferred to ArcMap and used to create vector images indicating placement of solitary traps as well as drift fences. Since the GPS took UTM measurements using the NAD27 datum, the trap vector layer had to be projected to NAD83 in order to line up with the other layers.

Data collected during my thesis research has been organized into Microsoft Access tables which are related to the above vector data sets. Separate tables for adult salamander collections, recaptured individuals, and egg masses of *A. texanum*, the species found during the study, have been developed. A one-to-many relationship has been created between adult captures and the trap database in ArcMap because several traps captured more than one individual. Egg masses and recaptured adults were added on their own respective layers.

## Chapter 3: Results

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### Ambystoma Found and Mark/Recapture Analysis

During preliminary searches of the study site 2 October 2004, one adult male *A. opacum* was found under a cover object. No nests or breeding adult *A. opacum* were found during autumn of 2004 or 2005, however five *A. opacum* larvae were captured with a dipnet on 15 November 2005 and three *A. opacum* larvae were trapped during spring 2005. No adult or larval *A. jeffersonianum* or *A. maculatum* were trapped, nor were any egg masses from these species found during the course of the study.

Between 12 February 2005 and 26 March 2005, 85 adult *A. texanum* were captured, measured and marked. Of the 85, 79 were from the main study site and six were from the supplementary study site. During that time, two *A. texanum* were recaptured at the main site. Using the revised Lincoln-Peterson mark/recapture formula, the mean population size is 503 and the standard deviation 231. This gives a maximum population estimate of 1428 to be used as the prior on the Poisson estimation. Figure 12 gives the results of this estimation and a population estimate of 605 to 700 individuals at the 95% confidence interval.

### Migration

No *A. texanum* from the main study site were found in the supplementary site during the study, nor were any from the supplementary site found in the main study site. This seems to indicate that the two sites are used by separate metapopulations. Since this could also be attributed to the small number of recaptures in general, more research including more trapping of the supplementary site and surrounding wetlands would be required to be certain. Another important discovery to the understanding of *A. texanum* migration in this wetland are the presence of three subadults, two in an evening search on October 18, 2004 and one on October 7, 2005, walking above ground during crepuscular hours.

### Morphometrics

Simple statistics of the morphometric data collected are shown in Table 1. Cloacal length was only measured in half of the individuals due to its wide variability during and after breeding; it



has been omitted from the additional analyses presented. Weight is the most variable of the metrics with the standard deviation being 25.3% of the mean. Snout-vent length is the least variable with a 9.2% coefficient of variance. Tail length is skewed toward the right, partially due to two individuals found with truncated tails. Weight is skewed to the left partially due to a pregnant female. These outlying individuals were not included in the comparisons.

Figure 13 shows histograms of each morphological metric, each split into 15 bins except for tail length which was split into 20 bins due to its outliers from individuals with truncated tails. Weight has a single outlier of a pregnant female at 22 grams; however this outlier still closely fits its skewed distribution. Each of the spatial metrics – cranial width, tail length, and snout-vent length – are bimodal, though snout-vent length is less so than the others.

Figure 14 shows these measurements plotted against each other. As expected, all trend lines have a positive slope – individuals with larger values in one metric tend to have larger values in other metrics as well. However, no predictive correlation was found between any of the morphological metrics.

Cranial width, snout-vent length, and tail-length were all very poorly correlated with one another. The best correlations were between the three spatial measurements and weight, with weight versus cranial width having  $R^2=0.44$ , snout-vent length  $R^2=0.38$ , and tail length  $R^2=0.28$ . When tail length and snout-vent length were summed to create an overall length and then compared to weight, a stronger correlation emerged ( $R^2=0.48$ ). Though stronger than other comparisons, even this correlation is not predictive, suggesting a large degree of variability in the population.

Although some information was repeated by collecting both spatial and weight metrics, because of the poor correlation, none of the metrics collected are capable of replacing any other. All metrics measured give additional information about individuals and the population as a whole.

## **Breeding Behavior**

Upon the evening of 1 March 2004 at approximately 10:00 pm, breeding masses of *A. texanum* were observed at the research site. Courtship begins with adult male and female salamanders amassing in the pool. Males swim over and under the females and use their heads to nudge the females. Males then swim to the bottom of the pool and deposit a cone shaped spermatophore on leaf litter. The female follows and shimmies her cloaca over the spermatophore, and uses her cloacal lips to nip off the top of the structure where the sperm are located. Breeding continued for several hours.

## **Clutch Size/Location**

Table 2 shows the location and size of the 17 egg masses found during the study. Eggs were deposited in masses ranging in size from four eggs to eleven eggs, with an average of six eggs per mass. All sites where eggs were laid shared common characteristics; most notably they were places of a medium depth averaging with lots of woody debris (Figure 15 and 16).

## **Larval Development**

Table 3 shows the location and sizes of the larvae found on capture dates. Few larvae were found during this investigation. This is likely due to the size of the wetland with the long furrows of standing water as well as the amount of woody debris providing excellent refugia for developing salamander larvae to hide, making capture of larvae difficult. Predation both by adult salamanders and by crayfish may have also played a factor, as they were often found in traps.

## **Mutations**

Several mutations were noted in individual *A. texanum* from both the main study site as well as the supplementary site during the course of study (Table 4). The head spot mentioned was a small round wound on the top of the cranial region approximately 4.5 mm in diameter that exposed the pinkish flesh underneath. Six out of the nine salamanders captured on that date in that trap exhibited this wound, but it was not encountered in any of the other individuals captured during the study.

## Chapter 4: Discussion

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### Ambystoma Found

Out of four Ambystomatids likely to be present within the study site, only evidence of *A. opacum* and *A. texanum* was discovered, no egg masses, larvae or adults of *A. maculatum* or *A. jeffersonianum* were found during the year long investigation. This suggests that either the species are not present or habitat partitioning is occurring, meaning that salamanders are altering activity and microhabitat usage to increase survivorship.

Many larval salamanders are intraguild predators of potential competitors, and if not, they still compete for a finite amount of habitat and resources. Thus, pond breeding salamander communities are shaped by density-dependent interactions among larvae. Brodman and Jaskula (2002) studied microhabitat use and activity patterns of 5 species of *Ambystoma* that commonly occur in ephemeral wetlands in Indiana: *A. tigrinum* (tiger salamander), *A. laterale* (blue-spotted salamander), *A. opacum*, *A. jeffersonianum*, and *A. maculatum*. The larvae were placed into laboratory ponds that were partitioned into microhabitats that either contained or lacked refugia.

The results of this study showed two interesting things. First, species with smaller larvae hid more frequently. And secondly when species were paired, both species changed their activity and microhabitat use. Brodman (1996) found similar results in a separate study with *A. maculatum* and *A. jeffersonianum*, with partitioning of the microhabitat and increased use of refugia when species were placed in the same experimental ponds. This may lower interspecific aggression and intraguild predation.

Since *A. opacum* larvae were found in the pond, adults must be using it as a breeding site. However, no nesting adults were found within the 25 search hours conducted during the study in either late 2004 or late 2005. I believe females may be nesting just underground in empty crayfish burrows or the abandoned burrows of shrews, voles, or other small mammals, which has not been discussed or addressed in other studies. This hypothesis is not outside the range of possibility because nest site selection by females has been shown to be influenced by microsite elevation within the pond bed, site hydrologic regime, cover availability, and soil moisture

(Petranka and Petranka, 1980; Petranka and Petranka 1981; Jackson et al., 1989; Figiel and Semlitsch, 1995; Wojnowski, 2000).

## **Morphometrics**

The bimodal distributions of the spatial metrics suggest that two subpopulations may be extant in the study sample—for example males/females or first year/second year adults. However, the lack of correlation between spatial metrics is inconsistent with this hypothesis. That is, although each metric has two ‘groups’, large and small, membership in a group is not consistent. This, however, leaves the causes of the bimodality unexplained.

Fiorentino (2002) found sexual dimorphism in SVL between males and females with females being the larger and Downs (1989) found a similar trend in the SVL of *A. texanum* from Ohio, which could account for the bimodality in SVL. This however, does not explain the bimodal trends in the other variables or why the bimodality is inconsistent between parameters. Sexing was not performed during this study, because once breeding is over, the cloacal swelling exhibited in males decreases considerably, making determining sex rather difficult in individuals captured after breeding.

In an unpublished study by Stewart and Loughman, *A. texanum* from the research site were measured in early 2004 as well as from other small populations along the Ohio River in early 2004 and early 2005. Tables 5, 6 and 7 show comparisons of the means of the measurements from the current study and Stewart and Loughman’s, with accompanying p-values from t-tests. Note the difference in tail length between the two years at the same site, which could be due to a new cohort reaching sexual maturity. New younger and smaller individuals joining the breeding migration would lower the mean tail length for that year. This could also explain the bimodality of in tail length; tail length is due to cohort and SVL is due to sexuality, thus also explaining the lack of consistency in modal membership. An alternate reason for this difference could be difference in research methodologies when measuring.

Also note the smaller average weight for salamanders measured in this study as compared to the ones measured at other sites in 2004. This could again be attributed to a new cohort reaching maturity that year in point pleasant or a difference in nourishment and fat deposits. More

measurement would be needed on other individuals for comparison as well as tail fat analysis to test this trend and hypothesis.

## Migration

There were no recaptures between the main site and the supplementary site. The two sites are a considerable distance from one another and a xeric forest mostly composed of *Pinus strobus* (white pine) grows between the two sites. They are however linked by a large low lying area that fills with water, so movement between the two sites is possible.

The lack of recaptures of adult *A. texanum* between the two sites may indicate the existence of a metapopulation, a set of subpopulations that are isolated from one another in which extinctions and colonization may occur. The main study site seems to be the “source” population that provides individuals to colonize the “sink” population at the supplementary site. In a study by Griffis and Jaeger in 1998 on a metapopulation of *Plethodon shenandoah* (Shenandoah salamander), territoriality from *Plethodon cinereus* (red-backed salamander) led to emigration of *P. shenandoah* to five small “sink” populations from the large “source” population (Griffis and Jaeger 1998). Conspecific or intraspecific territoriality may be leading *A. texanum* from the main site to colonize the supplementary site, but more data is needed before such a conclusion is made.

No migration into or out of the ponds was observed. However, two subadult *A. texanum* were found 18 October 2004 and another on 7 November 2005; both occasions were before the forest was inundated. This unseasonal occurrence of 1<sup>st</sup> year metamorphs may indicate that after metamorphosing, individuals burrow in wetland for summer and emerge the following fall and/or winter, a possibility which has not been discussed in the literature.

It may also be possible that after breeding, adults burrow underground within the dry areas of the wetland and that no migration into or out of the breeding wetland occurs. No adult migration either into or out of the breeding site was observed, no clear migration patterns were evident in the trapping data, nor were any adults found as roadkill on nearby highway 32, which seem to support this hypothesis. Due to the unique features of the study site like the raised ridges which occur between the water filled furrows, it would be possible for adult salamanders to return

underground while the wetland was still inundated. This possibility has not been addressed in other studies.

## **Breeding Behavior**

Wyman (1971) observed males dorsally amplex females before leading them to spermatophores in north-central Illinois. No amplexus or leading behavior was observed during breeding at the site. My observation is consistent with observations from Garton (1972) in southern Illinois and Licht and Bogart (1990) in Ontario. Time of breeding is also variable, as evidenced by breeding on 1 March in 2004 and in early February in 2005 as well as reports from other states of breeding dates (Minton, 1972, Brown et al. 1982, Downs, 1989, Minton 2001, Fiorentino 2002). Table 8 shows the reported ranges of breeding dates of *A. texanum* in various states. This variation makes sense if movements were cued by temperature and moisture as several authors have hypothesized (B.A. Brown et al., 1982; Petranka, 1984; Kraus and Petranka, 1989).

## **Clutch Size/Location**

Petranka (1982a) found that some populations in IL, IN, KY, OH, and TN lay eggs singly, and others in small masses (2-15). No single eggs were found in the study. Eggs were deposited in clusters of 4-11, with an average of about 6 eggs. This again seems to be a case of regional variation. It could also be attributed to the ease of finding larger egg masses as opposed to single eggs, though eggs layed in traps were also in clusters. Single eggs in traps could easily have been preyed upon by crayfish or adult salamanders. Fiorentino's study of *A. texanum* in Wood County, WV in 2002 found eggs in small clusters ranging from one egg to 35, with an average of 14 eggs per mass.

All sites where eggs were found had characteristics in common. They were all of a medium water depth with ample woody debris. These are the optimal sites for survival of eggs into larvae. Shallow sites are in danger of drying too quickly, while deeper sites may hold large predators. In fact, fish were trapped in traps on the fence located in the deepest part of the wetland (fence 3), two *Lepomis macrochirus* (Bluegill) and one *Luxilus cornutus* (Common Shiner). They were likely washed into the channel from the river or nearby streams during a winter flooding event.

Medium depth sites with lots of woody debris also provide more substrate for egg attachment as well as more refugia for larvae than sites that are deeper or shallower with less deadfall. These sites afford protection for developing larvae and habitat for macroinvertebrates which provide a plentiful food source for larval and juvenile salamanders. Some of the channels in the wetland exhibited a current at times and the deadfall would also provide a barrier to prevent eggs and larvae from being washed away.

## Mutations

Fiorentino (2002) documented mutations in six of the 24 individual *A. texanum* he captured in a breeding population in Boaz Swamp, Wood County, WV. These six salamanders each possessed one of three classifications of mutations: polyphalangy (extra digits), anophthalmia (missing eye), and ectrodactyly (missing digits). Since this represents 25% of capture individuals, Fiorentino states that the six *A. texanum* “represent the highest number of malformations in the state.”

In the current study, 11.76% of the individuals captured exhibited some form of malformation or serious injury. Mutations in adults are much less serious than those found in developing larvae and metamorphosing juveniles, which can reduce survivorship and negatively impact the population. The percent of individuals exhibiting malformations in Point Pleasant is a much less alarming figure than that of the Wood County population, though it is still important to discover what causes these mutations and when salamanders are exposed to these causes. Are these effects due to natural causes like failed predation and parasitic infection or anthropomorphic ones like high pollution levels? Additional research is required to resolve these questions.

Both tail truncation and missing digits can be due to failed predation. A large number of salamander predators were encountered in the wetland during the study (see the site description). Of particular interest are the crayfish. The loss of a portion of tail, toes or even feet could be attributed to encounters with crayfish. It has been well documented that *A. texanum* lives in crayfish tubes and uses them as refugia (Strecker and Williams, 1928; Cagle, 1942; Minton, 1972; Parmelee, 1993; Petranksa, 1998). Moreover crayfish were often trapped along with adult salamanders and the aggressive behavior was exhibited on these occasions. Due to the

burrowing habit of Ambystomatid salamanders, encounters with small burrowing mammals like shrews are likely, which might also account for the above injuries.

Missing digits might also be attributed to parasitic infection, as could malformed feet and the tumor-like growths that were observed. Studies show that larvae of parasitic trematodes burrow into developing amphibians, an intermediate host. The trematode infection forms cysts, which cause deformities. Johnson et al. (1999) found that community analysis of breeding pools with high rates of abnormal amphibians also support high numbers of aquatic mollusks, which are the first hosts of trematode parasites. Fingernail clams (*Sphaeriidae*), a common host of trematode parasites, were found both within the wetland and attached to the feet of several of the malformed salamanders.

The head spots found may be due to parasitic infection, fungal infection, or merely an open wound obtained from either conspecific aggression or during attempted escape from the trap. The latter is very unlikely however since six salamanders were observed with the sore in the same location, the top of the cranium where the head joins the neck. Bites and scrapes would occur lower on the head or on the snout and would also not exhibit the perfect circular form observed. Some form of communicable infection seems to be indicated because all six salamanders with the head spot were found in the same trap. The salamanders were shown to Dr. Tom Pauley, and Dr. Jim Joy (Marshall University); neither had seen anything like it before. Salamanders were released back to the point of capture when none of them seemed to be suffering adverse effects from the sore. Reasons for the wounds and the effect on adult salamanders are still a mystery.

Lastly it must be mentioned that pollution is also a known cause of mutations in salamanders. The location of the habitat would lend it to possible pollution from the residential area around the forest, the Ohio and Kanawha Rivers, the railroad, gas wells in the area, and ATV traffic through the wetland itself. All of these sources have been shown to harbor teratogenic compounds, which can cause developmental malformations in amphibians (Hopkins et al., 2000). Pesticides, fungicides, and fertilizers associated with agricultural and residential runoffs have been found to cause developmental anomalies in metamorphosing amphibians (Ouellet et al. 1997). To evaluate causes of the malformations, complete soil and water tests should be performed in the



area and individuals should be sampled for parasite analysis, all of which were outside the scope of the current study.

## **Final Conclusions**

As already discussed, salamanders are an integral part of forest food webs and energy flow in both the terrestrial and aquatic ecosystems. Also, due to their biphasic life cycles and skin permeability, amphibians are important indicators of general environmental health and may give early warning of harmful and polluted environments. Despite the lack of data on other *Ambystoma* species in this wetland, the population of *Ambystoma texanum* studied represents a large portion of the presence of this species in West Virginia. Preservation of this valuable breeding site is important to the conservation of this and other salamander species.

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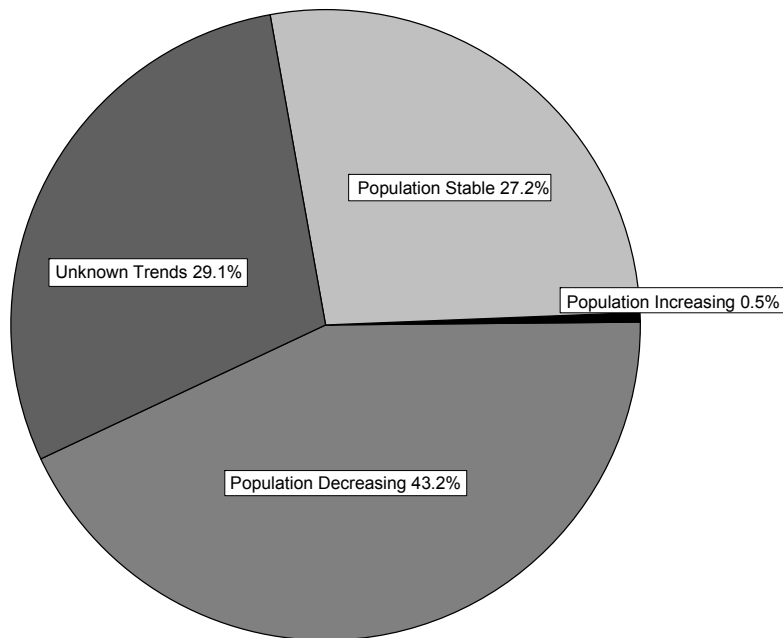
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## Appendix 1: Figures

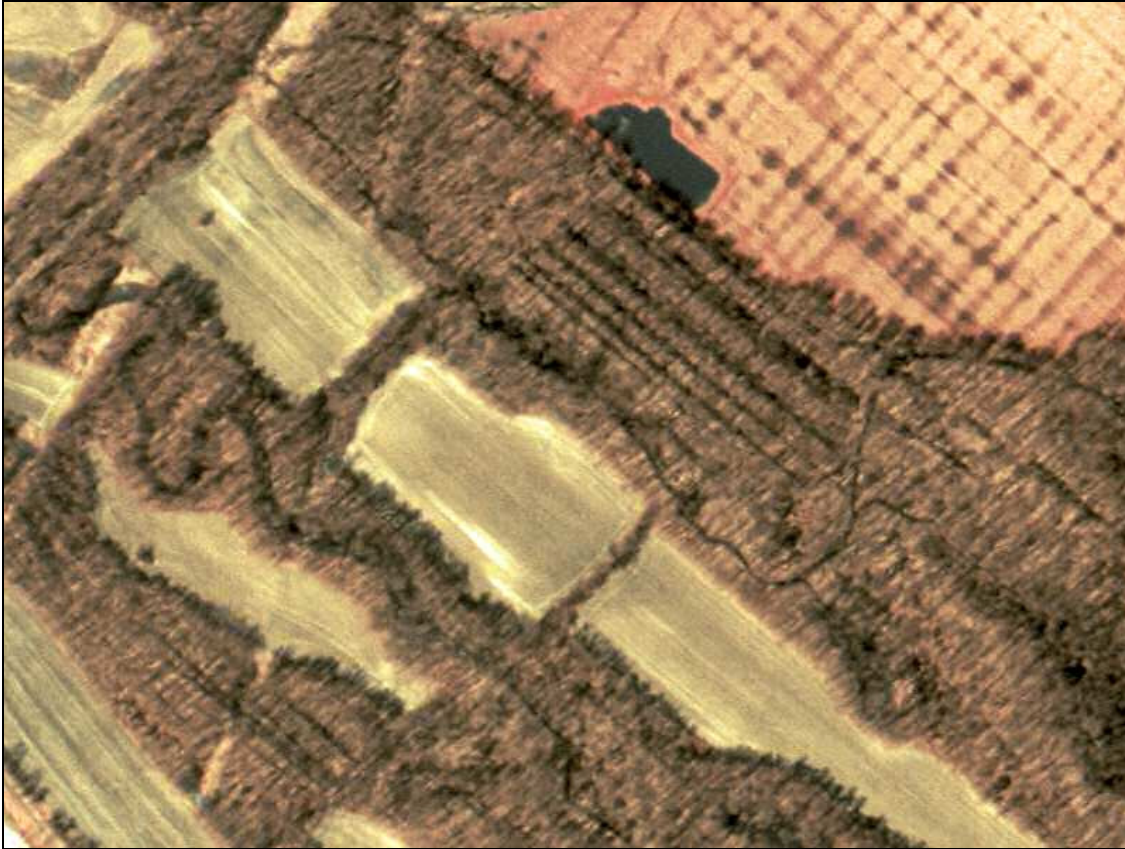
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**Figure 1.** Population dynamics of all amphibian species worldwide based on IUCN data from the Global Amphibian Assessment in 2004.



**Figure 2.** Aerial photo of study site. The road at the top of the image is highway ; the Moose Lodge parking lot is in the upper right corner. Source:



**Figure 3.** Digital Raster Graphic of the study site. Black areas are those with water. Source:





**Figure 4.** Adult *Ambystoma jeffersonianum*



**Figure 5.** Adult *Ambystoma opacum*



**Figure 6.** Adult *Ambystoma texanum*



**Figure 7.** Adult *Ambystoma maculatum*





**Figure 8.** Map of study site showing the location of traps and drift fences

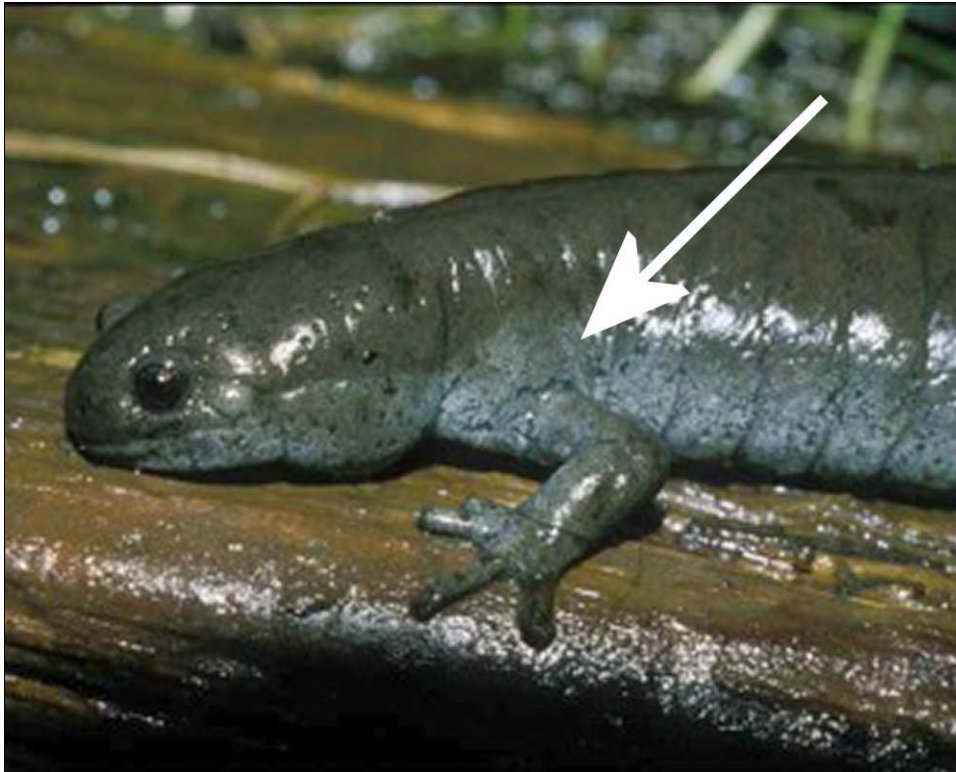


**Figure 9.** Drift fence with traps

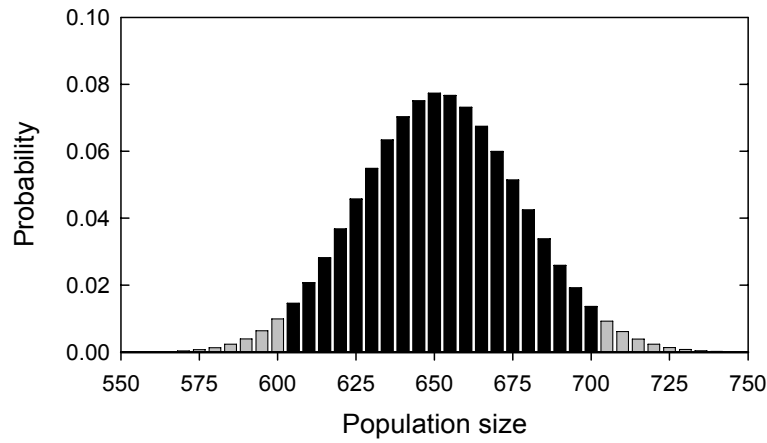




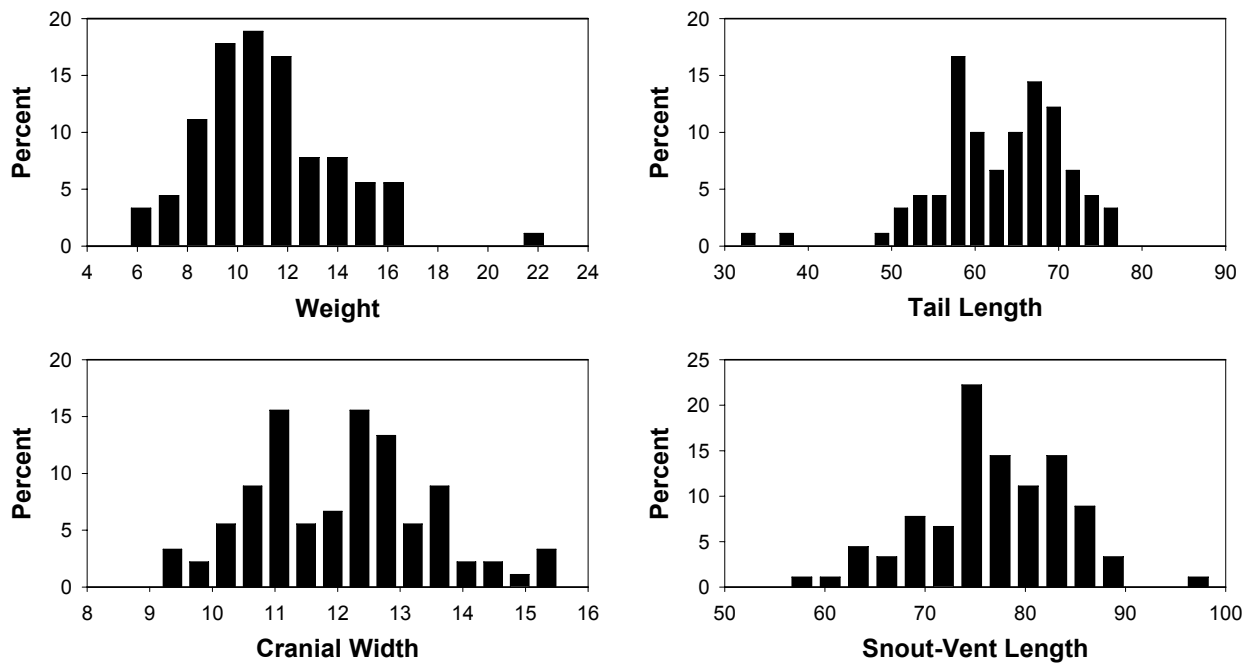
**Figure 10.** Drift fence with traps



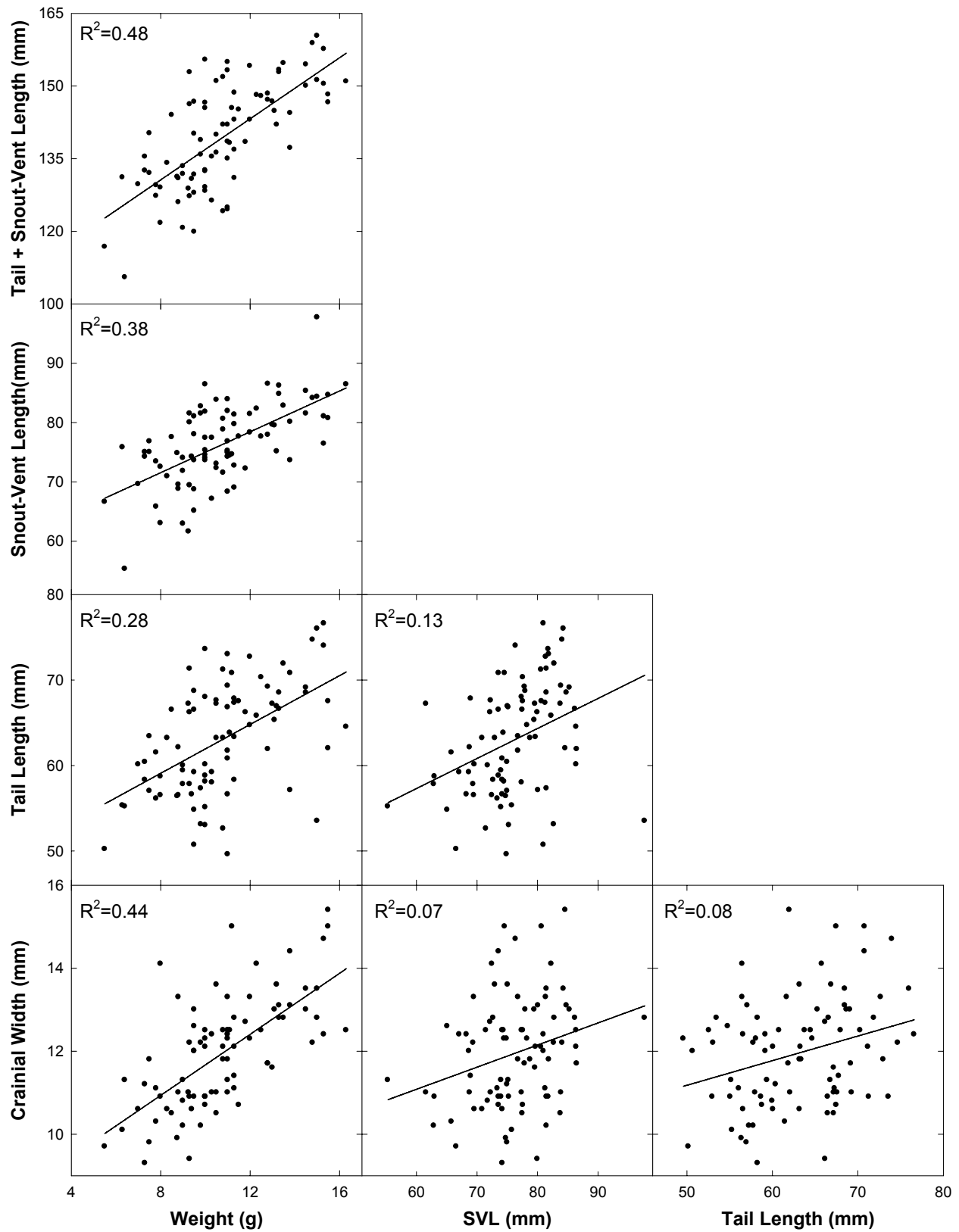
**Figure 11.** Photo of *A. texanum* showing visible implant elastomer marking site for individuals captured within the main study site.



**Figure 12.** Poisson probability distribution of *Ambystoma texanum* population size. Central black bars represent the 95% confidence interval.

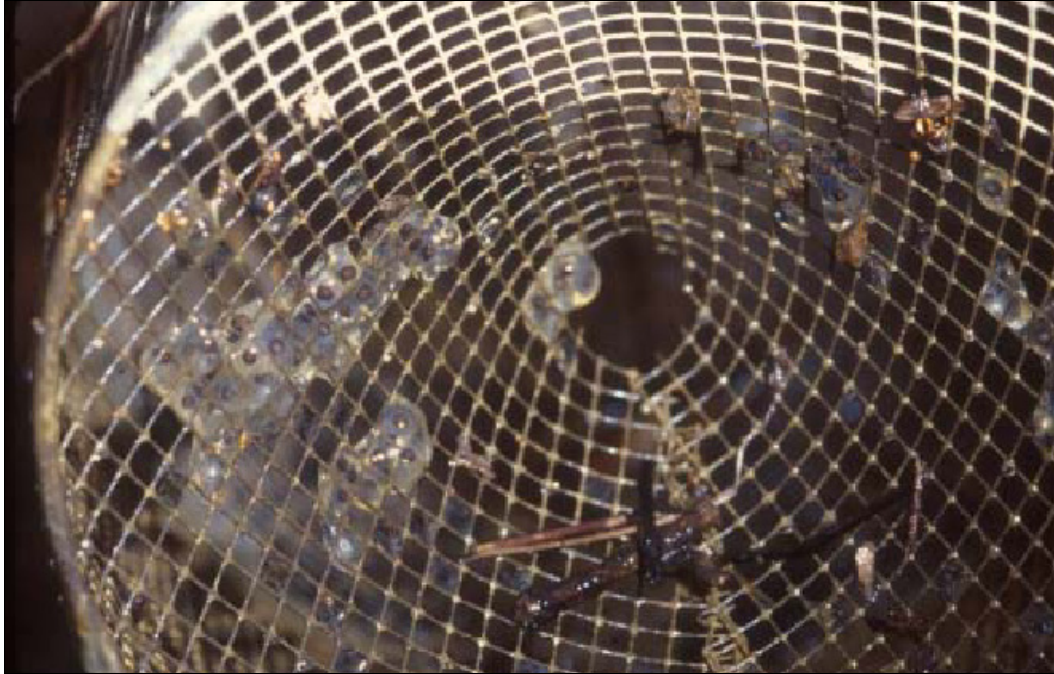


**Figure 13.** Histograms of morphometric data for 85 individual *Ambystoma texanum*.

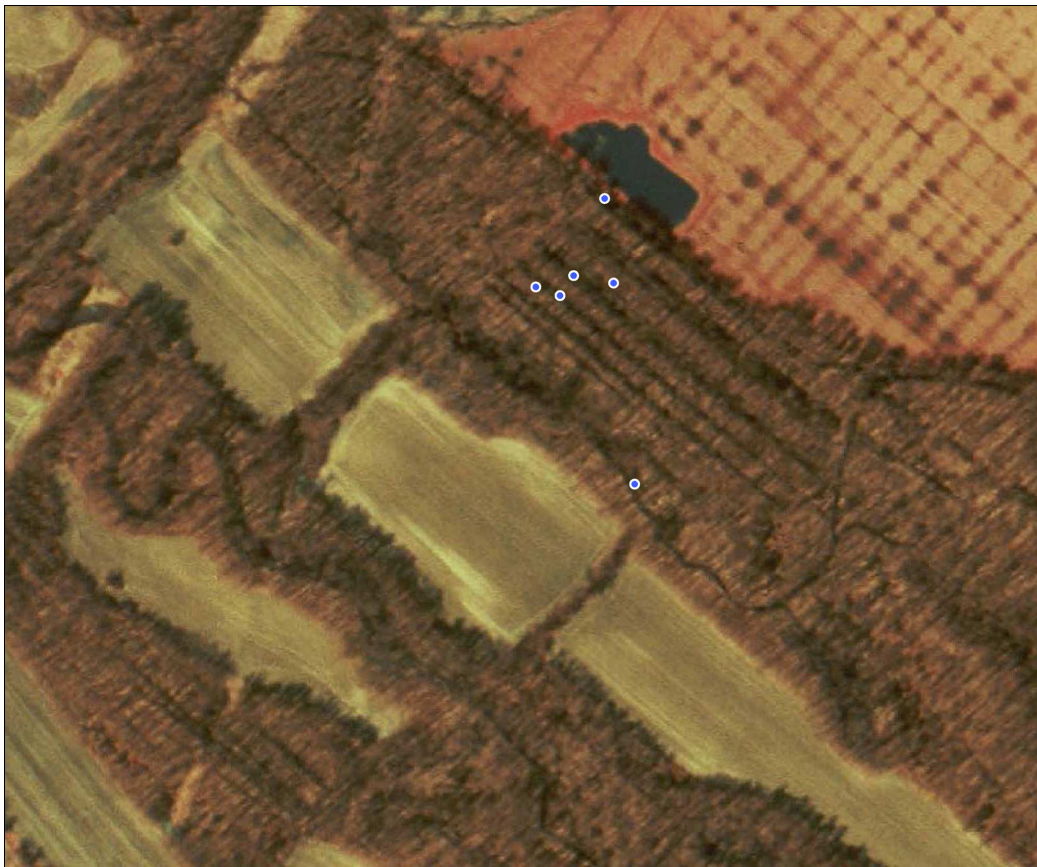


**Figure 14.** Morphological measurements of *A. texanum* plotted against one another showing trend lines and  $R^2$  values for each.





**Figure 15.** *Ambystoma texanum* egg masses. Photo by Robert Phipps and Robert Fiorentino.



**Figure 16.** Map showing locations of *Ambystoma texanum* egg masses

## Appendix 2: Tables

	Mean	Median	Std Dev	Std Err	Min	Max	Range	N	CV%
Weight (g)	10.77	10.5	2.72	0.29	5.2	22	16.8	90	25.3
CW (mm)	11.94	12.05	1.39	0.15	9	15.4	6.4	90	11.6
TL (mm)	62.23	63.2	7.87	0.83	30.9	76.6	45.7	90	12.6
SVL (mm)	75.95	75.55	6.98	0.74	55.3	97.7	42.4	90	9.2
CL (mm)	6.28	6.3	1.04	0.15	3.7	8.8	5.1	45	16.6

**Table 1.** Simple statistics of morphometric data collected from 85 individuals of *Ambystoma texanum*. CW: cranial width. TL: tail length. SVL: snout-vent length. CL: cloacal length.

Date	Location	Number of Eggs/Clutch
12-Feb-05	Sol-5	5
12-Feb-05	Sol-5	7
12-Feb-05	402656.583 E 4298655.582 N	6
12-Feb-05	402656.583 E 4298655.582 N	6
12-Feb-05	402656.583 E 4298655.582 N	7
12-Feb-05	402683.721 E 4298650.545 N	4
12-Feb-05	402683.721 E 4298650.545 N	5
12-Feb-05	402683.721 E 4298650.545 N	6
12-Feb-05	402683.721 E 4298650.545 N	7
27-Feb-05	F2-2	5
27-Feb-05	F2-2	6
27-Feb-05	F2-2	6
27-Feb-05	F2-2	8
27-Feb-05	Sol-6	11
27-Feb-05	F2-5	4
27-Feb-05	F2-5	7
27-Feb-05	F2-5	8
<b>Table 2.</b> <i>Ambystoma texanum</i> egg mass size and location		

Date	Method of Capture	Species	Size (mm)
15-Nov-04	Dip Net	<i>A. opacum</i>	20.5
15-Nov-04	Dip Net	<i>A. opacum</i>	22.4
15-Nov-04	Dip Net	<i>A. opacum</i>	24.8
15-Nov-04	Dip Net	<i>A. opacum</i>	21.6
15-Nov-04	Dip Net	<i>A. opacum</i>	25.3
9-May-05	F2-5	<i>A. texanum</i>	19.3
9-May-05	F2-5	<i>A. texanum</i>	22.4
9-May-05	F2-7	<i>A. texanum</i>	24.6
9-May-05	F2-7	<i>A. texanum</i>	20.5
9-May-05	F2-7	<i>A. texanum</i>	27.2
9-May-05	Sol-8	<i>A. texanum</i>	24.8
9-May-05	Sol-8	<i>A. texanum</i>	22.6
9-May-05	F1-2	<i>A. texanum</i>	26.6
9-May-05	F2-1	<i>A. opacum</i>	55.2
9-May-05	Sol-5	<i>A. opacum</i>	53.8
9-May-05	F1-8	<i>A. opacum</i>	56.4
<b>Table 3.</b> Location and Sizes of <i>Ambystoma texanum</i> and <i>Ambystoma opacum</i> larvae arranged by capture date.			

Date Captured	Location	Mutation
27-Feb-05	F3-1	tail truncated
27-Feb-05	Sol-5	tail truncated
26-Mar-05	F2-4	head spot
26-Mar-05	F2-4	head spot
26-Mar-05	F2-4	head spot
26-Mar-05	F2-4	head spot
26-Mar-05	F2-4	head spot
26-Mar-05	F2-4	head spot missing digits foot swollen
26-Mar-05	Sol-14	front left leg swollen missing digits
26-Mar-05	F2-5	tail tumor
<b>Table 4.</b> Mutations documented in <i>Ambystoma texanum</i> at study site and supplementary site		

Measurement	Pt. Pleasant 2005	Pt. Pleasant 2004	P value
Snout-Vent Length	75.95	74.33	0.25
Tail Length	62.23	71.62	< 0.01
Cranial Width	11.94	11.74	0.40

**Table 5.** Means of *Ambystoma texanum* measurements from the Point Pleasant site in 2005 (n=85) and 2004 (n=40) with accompanying p-values from t-Tests. Values less than 0.01 are significant.

Measurement	Pt. Pleasant 2005	Other Sites 2004	P value
Weight	10.77	14.94	< 0.01
Snout-Vent Length	75.95	89.14	0.17
Tail Length	62.23	63.96	0.80
Cranial Width	11.94	10.51	< 0.01

**Table 6.** Means of *Ambystoma texanum* measurements from the Point Pleasant site in 2005 (n=85) and other small populations in 2004 (n=10) with accompanying p-values from t-Tests. Values less than 0.01 are significant.

Measurement	Pt. Pleasant 2005	Other Sites 2005	P value
Snout-Vent Length	75.95	78.89	0.17
Tail Length	62.23	71.06	< 0.01
Cranial Width	11.94	12.54	0.05

**Table 7.** Means of *Ambystoma texanum* measurements from the Point Pleasant site in 2005 (n=85) and other small populations in 2005 (n=16) with accompanying p-values from t-Tests. Values less than 0.01 are significant.

State	Range of Breeding Dates	Studies
Alabama	February	Brandon, 1966
Illinois	late February—March	Cagle, 1942 Smith, 1961
Indiana	late January—late March	Hay, 1892 Minton, 1972 Minton, Jr., 2001. Brown et al., 1982
Iowa	mid-February—mid-April	Bailey, 1943 Camper, 1990
Kansas	late February—March	Plummer, 1977
Louisiana	mid-January	Doody, 1996
Ohio	Late February—March	Downs, 1989
Oklahoma	February—mid-March	Bragg, 1949
Texas	mid-January—February	Ramsey and Forsyth, 1950
West Virginia	February—early March	Fiorentino, 2002 Kaylor, 2006
<b>Table 8.</b> Reported ranges of breeding dates of <i>Ambystoma texanum</i> in various states.		

## Appendix 3: Listing

Gibbs sampler implemented in MatLab to determine composite probability distribution function

```
function X=poisTest(lam, lame, Q, step)
% X = poisTest(lam, lame, Q, step)
%
% lam: Poisson mean of distribution
% lame: exponential prior critical point (max population)
% Q: number of iterations
% step: the size of each step (typically 1)

if nargin < 4
    step = 1;
    if nargin < 3
        Q = 1000000;
    end
end

S = rand(1,Q);
U = rand(1,Q);
X = zeros(1,Q);
X(1)= lam;

k = lam^step;
b = exp((lam/lame)^step);

for i=1:(Q-1)
    x = X(i);

    if S(i)< .5
        y= x+step;
        r= k/prod((x+1):y)/b;
    else
        y= x-step;
        r= b*prod((y+1):x)/k;
    end

    if r > U(i)
        X(i+1) = y;
    else
        X(i+1) = x;
    end
end
```

	<pre>% assign initial values to % optional inputs  % create random lists  % initialize the stepper to the mean  % precalculations  % try a step forward % Poisson probability with prior at this step  % or try a step backward % same as above  % if the probability is greater than some % value on uniform(0,1), then accept % otherwise discard.</pre>
--	--